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Explorative study of anticoagulant activities of extracts of *Swietenia mahogany*, *Cissampellos mucronatta* and *Heckeria insignis*.

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ABSTRACT

The bark of Swietenia mahogany, the roots of Cissampellos mucronatta and the seeds of Heckeria insignis were extracted with water, methanol and carbon tetrachloride respectively. Each extract was filtered and concentrated under vacuum at 40°C and then were used as anticoagulant agents using whole blood clotting time method. All the control values obtained (5.4 minutes -5.9 minutes) lie within the reported normal range for blood coagulation time. The mean coagulation time obtained for all the extracts of roots of Cissampellos mucronatta were longer (6.0 minutes-7.5 minutes) than their controls but the difference was not significant. The water and methanol extracts of the bark of Swietenia mahogany and seeds of Heckeria insignis possess anticoagulant activities (the mean coagulation time observed for each of these extracts was more than 24 hours). However, the carbon tetrachloride extract did not show any significant anticoagulant activities. Preliminary phytochemical analysis showed that bark of Swietenia mahogany possesses terpenes and steroids whereas roots of Cissampellos mucronatta and seeds of Heckeria insignis possess tannins and saponins. These results are of interest because Cissampellos mucronatta and Heckeria insignis are used locally for treating snake bites and small cuts in some parts of Adamawa State, Nigeria.

INTRODUCTION

Plants normally produce many organic substances like phenolic compounds, coumarin compounds, terpenoids, steroids, alkaloids and many other phytochemical components. These chemical substances differ from one specie to the other, so that there is a wide variation of chemical substances in the plant kingdom. These chemical substances could be utilized for the treatment of diseases (Sofowora,1986 and Sofowora and Adebiyi,1987).

Snake bite has become a serious medical problem in some parts of the world especially in rural areas where the expensive conventional anti venom drugs are scarce and not affordable when available. The rural dwellers depend on plants for the treatment of snake bites. The traditional healers in Adamawa State, Nigeria use some plants for treatment of snake bites and minor cuts. They use bark of *Swietenia mahogany* for the treatment of snake bites. *Swietenia mahogany* (hausa name Madaci) belongs to the family of meliaceae. It is a timber tree which grows in all parts of Nigeria. The traditional healers also use roots of this plant for treatment of stomach pain and malaria (Sofowora, 1979). *C. mucronatta* (Hausa name Gwandan daji) belongs to the family menispermaceae and this plant is found almost everywhere in Nigeria where there is enough rain fall. Roots/tubers/rhizomes are used locally in traditional medicine to treat small cuts. *Heckeria insignis* (local name Bakore) belongs to the family piparaceae and these plants are abundant in Nigeria. In traditional medicine, the seeds of *H. insignis* is used in the treatment of snake bite (Kokwaro,1976). The aim of this work is to study the anticoagulant activities of water, methanol and carbon tetrachloride extracts of *S. mahogany, C. mucronatta* and *H. insignis* and to determine the phytochemical components of these plant materials used locally for curing some type of diseases.

MATERIALS AND METHODS

Preparation of samples

Bark of *S. mahogany,* roots of *C. microwatt* and dried seeds of *H. insignias* were collected from traditional practitioners in Gobi local Govt. in Adamawa State, Nigeria. The plants were identified and authenticated by School of Agriculture and Agricultural Technology, Federal University of Technology, Yola according to Keay and Onochie(1960). Fifty grams each of bark of *S. mahogany and* roots of *C. mucronatta* were cut into small pieces and then sun dried for 5-7 days. Then the dried samples were crushed using mortar and pastel and then into dried form using an electric blender. The micro ionized samples were kept for extraction purpose.

Preparation of extracts from micro ionized samples

Distilled water, methanol and carbon tetrachloride were employed as solvents for all the three samples. Water was used to simulate the conditions in which the herbalists generally use the herbs, methanol and carbon tetrachloride because of their broad spectrum and relative non-selective property of extracting photochemical components. Thirty grams of each of the powdered samples was extracted with 120 ml of distilled

Two grams of each of the powdered samples was added to 10 ml distilled water and boiled for 30 minutes. Then it was filtered and then 1 ml of 5% ferric chloride and 1 ml of 1% potassium ferric cyanide were added to the filtrate.

Effect of extracts on blood clotting time

A modification of the basic Lee and White method (Igboechi and Anufuro, 1986) for determination of whole blood clotting time was used. For this technique, the clotting time is the time required for a firm clot to be formed in fresh blood placed in glass tube containing the sample or control solvent. About 0.1 ml of each of nine samples and three controls was taken in a cleaned 13x10 pyrex test tube and 1 ml sample of fresh blood was allowed to run gently the side of the tube containing the control or sample to avoid bubbling before gentle mixing of blood and control/sample. For this purpose, fresh blood was drawn from human vein with a 20 gauge needle into a clean dry syringe. A stopwatch was started as soon as blood entered the syringe. The needle was removed and 1 ml of blood was immediately introduced into the tube containing sample or solvent. After gentle mixing, the tubes were then placed in a water bath at 37°C. The tubes in rotation were gently tilted every minute until one can be inverted without loss of contents. The remaining tubes were then tilted every 30 seconds. The coagulation time was recorded for each sample or solvent.

RESULTS

pH of the extracts

The pH of the extracts were within the range of 4.0- 5.0. The results are listed in Table 1.

Table1: pH of the concentrates of the extracts

*Extract	pH		
	Plants		
	H. insignis	*C. mucronatta*	*S. mahogany*
AQ	4.0	4.1	4.2
MT	4.3	4.7	4.0
CL	4.5	5.0	4.4

* AQ –aqueous extract, MT-methanol extract, CL- carbon tetra chloride extract.

water using a Soxhelet apparatus. Solvent extracts were filtered by using a table centrifuge and each of the extracts was then concentrated to 5 ml using a rotary evaporator. For extraction with methanol, 30 grams of each of the powdered samples was extracted with120 ml of 97% methanol using a Soxhelet apparatus. Solvent extracts were filtered and a rotary evaporator was used in vacuo at 40°C to concentrate, evaporate and recover the solvents. The concentrates (5ml each) were kept in refrigerator for further use. For extraction with carbon tetrachloride, the procedure was similar except that carbon tetrachloride was used instead of methanol. The concentrates were also kept in refrigerator for further use. The pH of each of the concentrates was determined using a pH meter.

Preliminary phytochemical analysis of bark of *S. mahogany*, roots of *C. microwatt* and dried seeds of *H. insignias*

A preliminary phytochemical analysis to screen the samples for the presence of phytochemical components namely tannins, terpenes, saponins and steroids was performed. This was performed according to Cuilei, 1982 and Sofowora,1986.

Tannins

Five gram ((5.0 g) of each of the powdered samples was mixed with 10 ml of distilled water and the solution was filtered. A few drops of 5% ferric chloride were added to the filtrate.

Saponins

About 0.5 g of each of the powdered samples was added to 10 ml of distilled water in a beaker and the mixture was warmed for 5 minutes and then 2 drops of 5% ferric chloride solution was added to it.

Terpenes

About 0.5 ml of aqueous extract of each of the samples was mixed with 0.1 ml of ethanol and was shaken for several times. About 0.4 ml of 5% H_2SO_4 containing 0.5% ferric chloride was added and stirred the contents with a glass rod.

Steroids

Preliminary phytochemical analysis

Tannins

The bark of *S. mahogany* did not show any green or blue green precipitate after the addition of ferric chloride. This observation indicates the absence of tannins in this sample. But in the case of roots of *C. mucronatta* and seeds of *H. insignis* the samples showed green precipitate after the addition of ferric chloride. This observation indicates the presence of tannins in these two samples (Table 2).

Table 2: Preliminary phytochemical analysis of plant samples

Plant	Component	Observations
H. insignis	Tannins	+
	Saponins	+
	Terpenes	-
	Steroids	-
C. mucronatta	Tannins	+
	Saponins	+
	Terpenes	-
	Steroids	-
S. mahogany	Tannins	-
	Saponins	-
	Terpenes	+
	Steroids	+

\+ active component present
\- active component absent

Terpenes

In case of the roots of *C. mucronatta* and seeds of *H. insignis*, the treated samples showed no change in colour after boiling. This shows the absence of terpenes in the samples. In case of S. mahogany, the treated sample showed change in colouration from colourless to pink. It indicates the presence of terpenes in the sample (Table 2).

Saponins

In case of bark of S. mahogany, the treated samples did not show any frothing which persisted on warming. But in case of seeds of *H. insignis* and the roots of *C. mucronatta*, the samples showed frothing which persisted on warming. This observation indicates the presence of saponins in these two samples (Table 2).

Steroids

In case of roots of *C. mucronatta* and seeds of *H. insignis*, the treated samples showed no change in colour after addition of potassium ferric cyanide. This shows the absence of steroids in the samples. In case of bark of *S. mahogany*, the treated sample showed blue colouration after addition of potassium ferric cyanide. This indicates the presence of steroids in the samples (Table 2).

Effects of extracts on blood clotting time

When whole blood clotting time was determined, the mean control values obtained for water, methanol are 5.4± 1.3 minutes and 4.9± 1.2 minutes respectively and for carbon tetrachloride the respective value was 5.7 ± 1.0 minutes. The mean coagulation time for all the extracts are listed in Table 3.

Table 3: Effect of extracts of the plants on whole blood clotting time

*Sample/Control	#mean (n ± SD) of clotting time		
	H. insignis	*C. mucronatta*	*S. mahogany*
Control 1	5.4± 1.3 minutes	5.4± 1.3 minutes	5.5± 1.4 minutes
Control 2	4.9± 1.3 minutes	4.9± 1.3 minutes	4.8± 1.3 minutes
Control 3	5.7± 1.3 minutes	5.7± 1.0 minutes	5.8± 1.1 minutes
AQ	> 24 hours	7.5± 1.1 minutes	> 24 hours
MET	> 24 hours	7.2± 1.3 minutes	> 24 hours
CAT	0.9± 1.1 minutes	6.8± 1.2 minutes	6.0± 1.5 minutes

*Control 1- water
Control 2-methanol
Control3- carbon tetrachloride
n (number of determinants) 12.

AQ- aqueous extract
MET- Methanol extract
CAT- carbon tetrachloride extract

DISCUSSION

Plants normally grow on different nature of soils which are extremely rich in microorganisms and infection remains a rare event. To keep out potential invaders, plants produce a wide range of selective antibacterial, antifungal and antiviral compounds either in a constitutive or an inducible manner (Cammue *et al.*, 1992). Among those compounds several low molecular weight proteins or peptides have been isolated in recent years from various plants and are believed to be involved in anticoagulant mechanism to prevent blood sample from clotting. Kone-bamba (1987) reported that *Physalis angulata* (faminy of Solanaceae) exhibited anticoagulant activities. Like aspirin, garlic has been shown to inhibit platelet clotting (and hence blood clotting) in isolated cell preparations. Ginkgo (*Ginkgo Biloba*) has also anticoagulatnt activities (Corrigan, 1995). Fucoidin, a homopolymer of sulphated L-fucose, isolated from the tissue of plants (*Stichopus japonicus*, *Ludwig-othurea grisea*), has been reported to have antithrombotic and anti-infective activities (Mulloy *et al.*, 2000; Gunay and Linhardt, 1999). The higher plants also contain sulphated polysaccharides- glycosaminoglycans (GAGs), which have activity heparin-like, and were isolated from *Filipendula ulmaria* and *Paeonia anomalia*, *Paeonia suffructicosa* (Liapina *et al.*, 1995; Liapina *et al.*, 2000). While checking anticoagulant activities in crude fractions from Wakan-Yakus (traditional herbal drugs), Hayakawa *et al.* (1995) detected antithrombin activity in the polysaccharide fraction of the leaves of *Artemisia princeps* Pamp. Bark of *S. mahogany,* roots of *C. mucronatta* and dried seeds of *H. insignis* were tested here to see whether these plant parts possess anticoagulant properties as claimed by some traditional herbalists in some parts of Nigeria. The mean coagulation time observed with aqueous, methanol and carbon tetrachloride extracts (7.5 minutes, 7.2 minutes and 6.5 minutes) of *C. mucronatta* were longer than their controls (5.4 minutes,4.9 minutes and 5.7 minutes) but the difference, however, not significant. Though the roots of *C. mucronatta* are used in some parts of Adamawa State as pain reliever and also for treating snake bites, the concentration used here may not be enough to act as anticoagulant agent. Results indicate that aqueous and methanol extracts *of H. insignis* and *S. mahogany* possess anticoagulant properties. The carbon tetrachloride extracts of *H. insignis* and bark of *S. mahogany* possess no anticoagulant properties at these concentrations used and the mean coagulation time for both the samples were longer than their controls. Preliminary phytochemical analysis shows that *H. insignis* possesses tannins and saponins whereas *S. mahogany* possesses terpenes and steroids. It also indicates that polar solvent extracts these phytochemical components effectively and it would seem that these phytochemical components in water and methanol extracts might be responsible for the *in vitro* anticoagulant properties activities of the extracts of the two plants. A similar pattern of result was obtained from the extracts of *Eupatorium odoratum*

and *Vernonia amygdalina* in which only water and methanol extracts possess anticoagulant activities (Igboechi and Anufuro,1986).

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